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(54) Title: METHOD OF MODIFYING CYTOTOXIC CELLS AND USES THEREOF

(57) Abstract

TALL–104 cells, and other cytotoxic T cell lines, may be modified to increase the cytotoxicity thereof, to enhance growth properties, and/or to provide a preferred phenotype, e.g., expression of cell surface antigens, function, e.g., change in cytokine production profile, by culturing the cells in an effective amount of IL–15, optionally followed by gamma irradiation to halt proliferation.

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METHOD OF MODIFYING CYTOTOXIC CELLS AND USES THEREOF

Field of the Invention

The invention relates generally to the modification of cytotoxic T cells by treatment with a selected cytokine, and to the use of such modified cells in cancer therapy.

Background of the Invention

The human T cell line TALL-104 ($CD3/TCR\alpha\beta^{+}CD8^{+}CD16^{-}$) [A. Cesano *et al*, In Vitro Cell. Dev. Biol., 28A:648 (1992); A. Cesano *et al*, J. Immunol., 151:2943-2957 (1993); and A. Cesano *et al*, Cancer Immunol. Immunoth., 40:139 (1995)] is endowed with MHC non-restricted killer activity and has been reported as useful, when lethally irradiated, against a broad range of tumors across several species, while sparing cells from normal tissues. As taught by the inventors' prior publications and patents cited above, unmodified TALL-104 cells are available from the American Type Culture Collection, 10801 University Boulevard Manassas, VA 20110-2209 under Accession Number CRL 11386 and are described in U.S. Patent No. 5,272,082. These cells may be preferably modified by lethal γ-irradiation and/or by stimulation in the cytokine Interleukin 2 (IL-2) or Interleukin 12 (IL-12) to provide them with an increased cytotoxicity against tumor and virus-infected targets.

Such modification methods have been described in detail in International Patent Publication No. WO94/26284, published November 24, 1994, which is incorporated by reference herein. For example, one modification step includes *in vitro* treatment of the TALL-104 cells with one or both of the two interleukins, recombinant human (rh) IL-2 and rhIL-12. When used independently to treat the cell line, IL-2 and IL-12 can induce the cell line's cytotoxic activity. When these cytokines are used together to modify the cell line, the modified cell line displays additive or increased

cytotoxic effects. This results in a significant increase in cytotoxic activity and recycling capability, ultimately leading to 100% elimination of tumor targets at an E:T ratio <0.1:1 [Cesano *et al*, J. Immunol., 151:2943 (1993)].

Another known modification step involves the exposure of the TALL-104 cell line to lethal irradiation to confer irreversible loss of growth capability with full retention of cytotoxic activity, both *in vitro* and *in vivo*. This is achieved by subjecting the cell line to γ-irradiation just prior to its use. Preferably, the cells are irradiated at 4000 rads using a ^{137}Cs source. As described in International Patent Publication No. WO94/26284, irradiation of TALL-104 cells provides a modified cytotoxic cell line that has lost its proliferative ability and, therefore, the possibility of growing in an unrestrained fashion in the recipient organism. These modified TALL-104 cells have been used in methods for the treatment of various cancers in humans and animals. See, also, US Patent Nos. 5,683,690; 5,702,702 and 5,820,856, and International patent publication No. WO98/48630, all incorporated herein by reference.

Other cytotoxic cells have also been described, such as the TALL-103/2 cells. See, US Patent No. 5,272,082 and A. Cesano *et al*, J. Immunol, 151:2943-2957 (1993); S. Visonneau *et al*, Cell Immunol., 165:252-265 (1995); and A. Cesano *et al*, J. Immunol., 160:1106-1115 (1998). However, TALL-103/2 cells, stimulated with IL-2 or IL-12, have been noted to have a limited spectrum of tumor target reactivities and display low levels of killing. These cells do not grow in severe combined immuno-deficient (SCID) mice. Thus, at present, TALL-103/2 cells have not appeared promising for clinical use.

Among the known cytokines, Interleukin-15 (IL-15) is a relatively novel T cell growth factor that shares some activities and receptor components with IL-2 [US Patent No. 5,747,024; J. G. Giri *et al*, J. Leuko. Biol., 57(5):763-6

(May 1995); L. S. Quinn *et al*, Endocrinol., 136(8):3669-72 (Aug. 1995)]. IL-15 utilizes the β and γ chains of the IL-2 receptor for signal transduction, but uses a different subunit (α) to bind to the cells. The expression pattern of IL-15 α receptor is distinct from that of IL-2 α receptor. IL-15 has been shown to induce LAK cell functions *in vitro* at high doses of about 100 ng/ml by a CD18-dependent, perforin-related mechanism [A. M. Gamero *et al*, Cancer Res., 55(21):4988-94 (Nov. 1995)]. IL-15 is produced by monocytes and dendritic cells and has been shown to induce cytokine production in human T helper cells, and adhesion receptor redistribution in T lymphocytes. It has been described to stimulate proliferation of γδ T cells and act synergistically with other stimuli in inducing lymphokine production thereby [See, also, W. E. Carson *et al*, J. Clin. Invest. 96(6):2578-82 (Dec. 1995); H. Jonuleit *et al*, J. Immunol., 158(6):2610-5 (Mar. 15, 1997); V. E. Garcia *et al*, J. Immunol., 160(9):4322-9 (May 1998); A. Mori *et al*, J. Immunol., 156(7):2400-5 (Apr. 1996); M. Nieto *et al*, Euro. J. Immunol., 26(6):1302-7 (June 1996); M. K. Kennedy *et al*, J. Clin. Immunol., 16(3):134-43 (May 1996)]. IL-15 has also been described as a vaccine adjuvant [US Patent No. 5,747,024], a therapeutic [US Patent No. 5,660,824], and an inducer of angiogenesis [A. L. Angiolillo *et al*, Biochem. Biophys. Res. Comm., 233(1):231-7 (Apr. 7, 1997)]. IL-15 has been said to have IL-2-like stimulating activities on T lymphocytes and NK cells [P. Allavena *et al*, J. Leuko. Biol., 61(6):729-35 (June 1997); J. P. DiSanto, Current Biol., 7(7):R424-6 (July 1, 1997); R. Evans *et al*., Cell. Immunol., 179(1):66-73 (Jul. 10, 1997)].

There exists a need in the art for methods for further enhancing the characteristics of cytotoxic T cells useful for therapy.

Summary of the Invention

In one aspect, the invention provides a method of modifying, or reversibly modifying, the phenotype and function of cytotoxic T cells while retaining the cytotoxicity of the cells comprising the steps of:

(a) culturing said cells in an effective amount of IL-15 thereby obtaining a high yield of a cell having a first phenotype;

(b) culturing the IL-15 stimulated cells in an effective amount of IL-2, thereby altering the first phenotype to a second phenotype; and

(c) optionally repeating steps (a) and (b) a selected number of times.

In another aspect, the invention provides a method of modifying a cytotoxic T cells while retaining the cytotoxicity of the cells comprising the steps of:

(a) culturing said cells in an effective amount of IL-2, thereby obtaining a first modified cell;

(b) culturing the IL-2 stimulated cells in an effective amount of IL-15; thereby obtaining a second modified cell; and

(c) optionally repeating steps (a) and (b) a selected number of times.

The first and second modified cells from either method above demonstrate a change in at least one characteristic, such as increased proliferation, differentiation, growth, phenotype, adhesion molecule expression, biodistribution, cytokine production profile, level of cytotoxic activity, and tumor target spectrum. Desirably the cells are TALL-104 cells or TALL-103/2 cells.

In one embodiment of the first method, TALL-104 cells are cultured in an effective amount of IL-15, wherein said cells grow at a rate faster than when stimulated by IL-2, and have an altered phenotypic profile; and then the IL-15 stimulated TALL-104 cells are cultured in an effective amount of IL-2. In an embodiment of the second embodiment, the

modification of cell characteristics is accomplished by first culturing TALL-104 cells in an effective amount of IL-2 and then culturing the IL-2 stimulated TALL-104 cells in an effective amount of IL-15.

In yet another aspect, the invention provides a method of modifying TALL-104 cells comprising culturing TALL-104 cells in an effective amount of IL-15, wherein said cells grow at a rate faster than when stimulated by IL-2, and have an altered phenotypic, cytotoxic and cytokine profile. The modified cells have an increased level of cytotoxicity or another change in a characteristic such as increased proliferation, differentiation, growth, phenotype, adhesion molecule expression, biodistribution, cytokine production profile, and tumor target spectrum. In one embodiment of this method the cytokine profile includes increased expression of IL-10, GM-CSF, TNF-α and TNF-β and decreased expression of gamma interferon (IFN-γ) by the modified TALL-104 cells. In another embodiment, the modified phenotype includes increased expression of the cytotoxic adhesion/activation marker CD56 and/or decreased expression of the adhesion molecule CD38.

In still another aspect, the invention provides a method for increasing the levels of cytotoxic activity and spectrum of tumor target recognition of TALL-103/2 cells comprising culturing TALL-103/2 cells in an effective amount of IL-15, wherein said cells grow at a faster rate and have an expanded tumor target spectrum of cytotoxicity than when stimulated by IL-2.

In yet a further aspect, the invention provides modified TALL-104 cells, which are produced by stimulating said cells in an effective amount of IL-15.

In another aspect, the invention provides modified TALL-103/2 cells having an increased cytotoxicity, which are produced by stimulating said cells in an effective amount of IL-15.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 is a graph which illustrates that IL-15 supports greater TALL-104 cell proliferation *in vitro*. The symbol □ indicates the 5 ng/ml dosage of IL-15; the symbol ◇ indicates the 100 U/ml dosage of IL-2. Growth of cells is measured by a metabolic surrogate marker, lactate (mg/dl), over days in culture.

Fig. 2 is a bar graph illustrating that upon expansion of TALL-104 cells in vivo in SCID mice and re-adaptation to tissue culture conditions, IL-15 induces quicker differentiation of TALL-104 cells into cytotoxic cells in comparison to the effects of IL-2. Undifferentiated TALL-104 cells, extracted from SCID mouse spleens, were cultured for one week with either IL-2 or IL-15, and then tested for cytotoxicity against either K562 or Raji tumor cells. Cytotoxicity is demonstrated by percent lysis of the tumor cells. The open bar indicates IL-2 treatment; the strippled bar indicates IL-15 treatment. TALL-104 cells stimulated in IL-15 also show higher levels of cytotoxic molecules such as perforin, serine esterases (SE) and TIA-1, an apoptosis inducing molecule.

Fig. 3 is a graph indicating that IL-15 induces higher expression of cytotoxic adhesion/activation marker CD56, both as percent of positive cells in the total TALL-104 population and at the single cell level, as the number of molecules present on each cell (higher density). The symbol □ indicates IL-2; the symbol ◇ indicates IL-15. Results are plotted as % CD56+ cells over time (days) in culture.

Fig. 4 is a graph demonstrating that undifferentiated TALL-104 cells freshly obtained from the SCID mouse have a higher expression of CD2 and that with time in culture CD2 surface levels decline. However, this decline

is slower in TALL-104 cells grown in IL-15 than in IL-2. The symbol □ indicates IL-2; the symbol ◊ indicates IL-15. Results are plotted as % CD2+ cells over time (days) in culture.

Fig. 5 is a graph demonstrating that TALL-104 cells grown in IL-15 have lower expression of the adhesion molecule CD38. The symbol □ indicates IL-2; the symbol ◊ indicates IL-15. Results are plotted as % CD38+ cells over time (days) in culture.

Fig. 6 is a bar graph illustrating the comparative effects of IL-15 and IL-2 on the induction of GM-CSF from TALL-104 cells. On the X axis are the stimuli used to trigger GM-CSF production. The graph shows that TALL-104 cells grown in IL-15 have a baseline level of GM-CSF production, and respond to OKT3 monoclonal antibody with higher production of GM-CSF than cells grown in IL-2.

Fig. 7 is a bar graph illustrating the comparative effects of IL-15 and IL-2 on the induction of IL-10 from TALL-104 cells. On the X axis are the stimuli used to trigger IL-10 production. The graph shows that TALL-104 cells grown in IL-15 have a baseline level of IL-10 production, and respond to OKT3 monoclonal antibody with higher production of IL-10 than cells grown in IL-2.

Fig. 8 is a bar graph illustrating the comparative effects of IL-15 and IL-2 on the induction of TNF-α from TALL-104 cells. On the X axis are the stimuli used to trigger TNF-α production. The graph shows that TALL-104 cells grown in IL-15 have a baseline level of TNF-α production, and respond to OKT3 monoclonal antibody with higher production of TNF-α than cells grown in IL-2.

Fig. 9 is a bar graph illustrating the comparative effects of IL-15 and IL-2 on the induction of TNF-β from TALL-104 cells. On the X axis are the stimuli used to trigger TNF-β production. The graph shows that TALL-104

cells grown in IL-15 have a baseline level of TNF-β production, and respond to OKT3 monoclonal antibody with higher production of TNF-β than cells grown in IL-2.

Fig. 10 is a bar graph illustrating the comparative effects of IL-15 and IL-2 on the induction of IFN-γ from TALL-104 cells. On the X axis are the stimuli used to trigger IFN-γ production. The graph shows that TALL-104 cells grown in IL-15 have a baseline level of IFN-γ production, and respond to OKT3 monoclonal antibody with lower production of IFN-γ than cells grown in IL-2.

Fig. 11 is a graph which illustrates that IL-15 supports the growth of TALL-103/2 cells in culture. The symbol □ indicates the dosage of IL-2 in U/ml; the symbol ◇ indicates the dosage of IL-15 in ng/ml. Growth of cells is indicated by cpm in ^{3}H-TdR proliferation assays. The X axis shows the concentration of the cytokines.

Fig. 12 is a bar graph demonstrating that IL-15 (strippled bars) supports the cytotoxic phenotype of TALL-103/2 cells and broadens the spectrum of target recognition by these cells. The cells were cultured for a week in either IL-15 or IL-2 (clear bars), and then exposed to K562 tumor cells (sensitive target) and H160 or Daudi tumor cells (resistant targets). Cytotoxicity is measured by % lysis of the target cells in ^{51}Cr release assays.

Fig. 13 is a bar graph which illustrates the comparative effect of IL-2 and IL-15 on the induction of IFNγ in TALL-103/2 cells. The X axis shows the cytokine dosage. Results show that IL-2 is a better inducer of this cytokine.

Fig. 14 is a bar graph which illustrates the comparative effect of IL-2 and IL-15 on the induction of TNF-β in TALL-103/2 cells. The X axis shows cytokine dosage. IL-15 induces higher levels of TNF-β at the concentration of 10 µg/ml.

Fig. 15 is a bar graph which illustrates the comparative effect of IL-2 and IL-15 on the induction of TNF-α in TALL-103/2 cells. Similar levels of TNF-α are induced by the two cytokines.

Fig. 16 is a bar graph which illustrates the comparative effect of IL-2 and IL-15 on the induction of IL-10 in TALL-103/2 cells. IL-15 is a better inducer of IL-10 as compared to IL-2.

Detailed Description of the Invention

Cytotoxic T cell lines, such as TALL-104, have found use in clinical settings, such as the treatment of cancers, when administered *in vivo*, or when employed in *ex vivo* therapeutic regimens. Still other cytotoxic T cell lines, such as TALL-103/2 could be clinically useful if their target specificity was broadened and their growth in culture improved. The inventors have now discovered novel methods for increasing the cytotoxicity of these cells, altering their phenotypes and spectrum of target recognition, and, increasing their yield in culture.

Such modifications can be introduced to TALL-104 or TALL-103/2 cells, and are anticipated to be introduced to other cytotoxic T cells by stimulating the cells in IL-15, rather than, or in addition to, IL-2. Such IL-15 stimulation may optionally be followed by exposing the stimulated TALL-104 cell line to lethal irradiation to confer irreversible loss of growth capability with retention of cytotoxic activity, both *in vitro* and *in vivo*. This may be achieved by subjecting the cell line to γ-irradiation just prior to its use. Preferably, the cells are irradiated at 4000 rads using a ^{137}Cs source, similar to the process described in International Patent Publication No. WO94/26284. Such irradiation of the IL-15 stimulated TALL-104 cells provides a modified cytotoxic cell line that has lost its proliferative ability and, therefore, the possibility of growing in an unrestrained fashion in the recipient organism.

Thus, in one embodiment, TALL-104 cells are prepared as follows. TALL-104 cells (ATCC CRL 11386) are exponentially grown in tissue culture in the presence of recombinant human (rh) IL-15. The resulting proliferation of the cytokine stimulated TALL-104 cells (as measured by ^{3}H-TdR uptake) is higher at plateau doses of IL-15 than at plateau doses of IL-2. A "plateau dose" is the dose at which maximal activity is reached, e.g., the optimal dose (see Fig. 1). These cells also demonstrate increased ability to adhere to plastic *in vitro* (and potentially to endothelium *in vivo*) by increase in expression of adhesion molecules. These modified TALL-104 cells also demonstrate increased cytotoxic function, as shown by higher levels of killing, increased spectrum of tumor target recognition, and a quicker and more effective kinetic of induction of lytic proteins, such as PFP, SE1 and SE2, and TIA1. Cells grown in an optimal dose of IL-15 generally show higher levels of cytotoxic activity, as compared to the same cells in an optimal dose of IL-2 (see Fig. 2, which demonstrates a significant increase in cytotoxicity against NK-sensitive K562 cells and NK-resistant Raji cells, as compared to the same TALL-104 cells stimulated in IL-2). The cells also demonstrate an increased expression of the cytotoxic adhesion marker CD56 (Fig. 3). TALL-104 cells grown in IL-15 have higher baseline levels of cytokines and respond to stimuli, such as antibodies and target cells, producing higher levels of cytokines than TALL-104 grown in IL-2, with the exception of gamma interferon (IFN-γ), which is produced in higher levels by stimulation of TALL-104 cells in IL-2. The same results were obtained with TALL-103/2 cells.

Therefore, according to one embodiment of this invention, TALL-104 cells may be grown in IL-15 simply to increase the yield thereof, and then grown in IL-2 to reproduce the IL-2 cytotoxic phenotype previously used in clinical therapies for cancer. Alternatively, one may grow TALL-104 cells in IL-15 and use the IL-15 phenotype where

enhanced adhesion to endothelium is desired in some clinical applications. The inventors have determined that one may reversibly switch the IL-15 and IL-2 phenotypes of TALL-104 by sequential growth of the cells in one and then the other of these two cytokines, as desired. The biodistribution of the TALL-104 cells may also be affected differently by the two cytokines, based on the different levels of expression of adhesion molecules.

In yet another embodiment of this invention, TALL-103/2 cells may also be modified by stimulation in IL-15. In this instance, the TALL-103/2 cells will grow more rapidly in culture (Fig. 11) when stimulated with IL-15. Most significantly, when TALL 103/2 cells are stimulated in IL-15, their target recognition expands, and these cells may then be used against more tumor cell types. For example, Fig. 12 shows the results of stimulation of TALL 103/2 cells with IL-15 vs. IL-2. The IL-15 TALL 103/2 cells are able to recognize and kill HL60 and Daudi cells, against which the IL-2 stimulated TALL 103/2 cells were not cytotoxic. Additionally, the stimulation of the TALL 103/2 cells with IL-15 alters the cytokine production by the cells. See, for example, Figs. 12-16, which showing cytokines that are likely to be involved in the anti-tumor activity of the killer cells. Thus, changing the cytokine profile can result in clinical changes, both in terms of efficacy and/or toxicity.

Based on the effects that IL-15 has on these two cytotoxic T cell lines, it is anticipated that similar effects may be obtained with other cytotoxic T cell lines. Thus, IL-15 may be employed in a method for reversibly altering the phenotype of cytotoxic T cells by culturing said cells in IL-15, thereby obtaining a high yield of a cell having a first phenotype; followed by culturing these cells in IL-2, thereby altering the first phenotype to a second phenotype. The second phenotype may be returned to the first phenotype by further culturing in IL-15 again, if desired. The IL-15 phenotypes are characterized by enhanced growth

kinetics, increased cytotoxicity, enhanced cytokine production, and, likely, increased adhesion to vasculature.

These IL-15 stimulated cytotoxic T cells may then be employed in methods for *in vivo* and *ex vivo* therapy of cancer, and for other uses for which TALL-104 cells are known, as described in the US patents incorporated by reference above. These modified cells may also be employed as research reagents, as reagents for screening the effect of proposed developmental drugs on their cytotoxic activity, as reagents for the study of their expression of adhesion molecules or cell surface markers, as well as for the production of cytokines or other biological molecules expressed by the modified cells.

The following examples demonstrate the effect of IL-15 on TALL-104 cells and TALL-103/2 cells. These examples illustrate the preferred methods of the invention. These examples are illustrative only and do not limit the scope of the invention.

EXAMPLE 1 - GROWTH OF TALL-104 CELLS IN IL-15

TALL-104 cells were grown in endotoxin-free Iscove's modified Dulbecco's medium (Gibco-BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Sigma) and 100 U/ml rhIL-2 (Chiron Therapeutics, Emeryville, CA) or rh IL-15 (1-5 µg/ml) [R& D Systems] in a humidified incubator at 37°C with 10% CO_2 in tissue culture for two weeks.

The cells were then examined for differences in characteristics such as growth, phenotype, cytokine profile, and cytotoxicity, biodistribution and tumor target spectrum of the cultures, and the results were reported in Figs. 1-10.

A. *Proliferation*

As shown in Fig. 1, TALL-104 cells grown in the plateau dose (5 ng/ml) of IL-15 proliferate *in vitro* faster than do the same cells grown in the plateau dose (100 U/ml) of IL-2.

B. Target Spectrum

In another experiment, the IL-2- or IL-15-treated TALL-104 cells were expanded *in vivo* in SCID mice and re-adapted to tissue culture conditions. IL-15 induced quicker differentiation of TALL-104 cells into cytotoxic cells in comparison to the effects of IL-2. Undifferentiated TALL-104 cells, extracted from SCID mouse spleens, were cultured for one week with either IL-2 or IL-15, and then tested for cytotoxicity against either K562 or Raji tumor cells. The IL-2-treated TALL-104 cells were only marginally (≤ 10%) cytotoxic, as demonstrated by percent lysis of the tumor cells, for K562 cells. In contrast, the TALL-104 cells stimulated with IL-15 lysed about 40% more K562 tumor cells. The IL-2-treated cells lysed no Raji cells, whereas the IL-15 treated TALL-104 cells lysed almost 60% of these cells. See Fig. 2. The IL-15 treated cells also showed higher levels of cytotoxic molecules, such as perforin, serine esterases (SE) and TIA-1, an apoptosis inducing molecule.

C. Phenotype

TALL-104 cells treated with IL-15 express higher levels of the cytotoxic/adhesion/activation marker CD56, both as percent of positive cells in the total TALL-104 population and at the single cell level, as the number of molecules present on each cell (higher density), than do TALL-104 cells treated with IL-2. See Fig. 3.

Undifferentiated TALL-104 cells freshly obtained from the SCID mice have a high expression of CD2. With time in culture, the CD2 surface levels decline. However, this decline when compared for TALL-104 cells grown in IL-2 or IL-15 as described above, was demonstrated to be slower in the IL-15 stimulated cells. See Fig. 4.

TALL-104 cells grown in IL-15 were also shown to have lower expression of the adhesion molecule CD38 than TALL-104 cells grown in IL-2. See Fig. 5.

D. Cytokine Profile

The TALL-104 cells, grown in either IL-2 or IL-15 as above, were stimulated to trigger cytokine production with OKT3 (anti-CD3), Moon-1 (anti-CD31), or IB4 (anti-CD38) monoclonal antibodies or by exposure to K562 cells.

As seen in Fig. 6, TALL-104 cells grown in IL-15 have a baseline level of GM-CSF production, and respond to OKT3 monoclonal antibody with a significantly higher production of GM-CSF than cells grown in IL-2.

As seen in Fig. 7, TALL-104 cells grown in IL-15 have a baseline level of IL-10 production, and respond to OKT3 monoclonal antibody with significantly higher production of IL-10 than cells grown in IL-2.

As seen in Fig. 8, TALL-104 cells grown in IL-15 have a baseline level of TNF-α production, and respond to OKT3 monoclonal antibody with higher production of TNF-α than cells grown in IL-2.

As seen in Fig. 9, TALL-104 cells grown in IL-15 have a baseline level of TNF-β production, and respond to OKT3 monoclonal antibody with higher production of TNF-β than cells grown in IL-2.

As seen in Fig. 10, TALL-104 cells grown in IL-15 have a baseline level of IFN-γ production, and respond to OKT3 monoclonal antibody with lower production of IFN-γ than cells grown in IL-2.

EXAMPLE 2 - GROWTH OF TALL-103/2 CELLS IN IL-15

TALL-103/2 cells were grown in endotoxin-free Iscove's modified Dulbecco's medium (Gibco-BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Sigma) and 100 U/ml rhIL-2 (Chiron Therapeutics, Emeryville, CA) or rh IL-15 (1-5 µg/ml) [R&D Systems] in a humidified incubator at 37°C with 10% CO_2 in tissue culture for two weeks. The cells were then examined for differences in

characteristics such as growth, phenotype, cytokine profile, and cytotoxicity, biodistribution and tumor target spectrum of the cultures, and the results were reported in Figs. 1-10.

A. Phenotype

The surface phenotype of the cultured cells were compared to determine the effect of the two cytokines. The results are reported below in Table I as % positive cells; the mean fluorescence intensity (on a scale with the upper limit of 200) is in parentheses and provides an indication of the antigen density, i.e., the number of molecules/cell.

TABLE I

Cell Surface Antigen	**IL-2 Treated TALL-103/2**	**IL-15 Treated TALL-103/2**
CD3	96.5 (92)	35.6(62)
CD2	53.5(75)	43.5(61)
CD4	4.4(60)	15(62)
CD8	90.1(131)	78.9(113)
CD56	41.6(60)	67.4(123)
LFA-3	99.3 (125)	88(118)
ICAM-1	71.5(84)	44.9(81)
CD45RO	81.3(83)	72.2(86)
CD38	70.5(74)	32.5(62)
CD31	35.6(61)	2.3(66)

B. Proliferation

In ^{3}H-TdR proliferation assays, TALL-103/2 cells grown in IL-15 showed greater proliferation than the cells grown in IL-2 at stimulating cytokine doses greater than 1 ng/ml IL-15. See Fig. 11.

C. Target Spectrum

TALL-103/2 cells were cultured for a week in either IL-15 or IL-2, and then exposed to K562 tumor cells, HL60 tumor cells or Daudi tumor cells. Cytotoxicity was measured by % lysis of the target cells in ^{51}Cr release assays. As demonstrated in Fig. 12, the IL-15 treated cells caused lysis of all three tumor cell types. The IL-2 treated cells were cytotoxic only for the K562 cells. Thus, the method of this invention supported the cytotoxic phenotype of TALL-103/2 cells and broadened the spectrum of target recognition by these cells.

D. Cytokine Profile

The IL-2-treated and IL-15-treated TALL-103/2 cells were also evaluated for dose-dependent cytokine production. As revealed by Figs. 13-16, the IL-15 induces production of cytokines from the cells, which is different from that produced by stimulating the cells with IL-2.

IL-2 stimulation induces better expression of IFNγ in TALL-103/2 cells, than does IL-15 stimulation (Fig. 13).

IL-15 induces higher levels of TNF-β at the concentration of 10 μg/ml (Fig. 14), than does IL-2 stimulation.

Similar levels of TNF-α are induced by the two cytokines (Fig. 15).

IL-15-stimulated TALL-103/2 cells produce greater amounts of IL-10 at concentrations over 10 ng/ml IL-15 (Fig. 16).

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the methods of the present invention are believed to be encompassed in the scope of the claims appended hereto.

Applicant's or agent's file reference number	WST88PCT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 10, line 2

B. IDENTIFICATION OF DEPOSIT TALL-104 cell lines Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209 USA

Date of deposit	Accession Number
June 15, 1998	CRL 11386

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer MISTY WALKER INTERNATIONAL DIVISION (703) 305-3682	Authorized officer

Form PCT/RO/134 (July 1992)

WHAT IS CLAIMED IS:

1. A method of modifying cytotoxic T cells while retaining the cytotoxicity of the cells comprising the steps of:

(a) culturing said cells in an effective amount of IL-15;

(b) culturing the IL-15 stimulated cells in an effective amount of IL-2; and

(c) optionally repeating steps (a) and (b) a selected number of times, wherein said modified cells demonstrate a change in at least one characteristic selected from the group consisting of increased proliferation, differentiation, growth, phenotype, adhesion molecule expression, biodistribution, cytokine production profile, level of cytotoxic activity, and tumor target spectrum.

2. The method according to claim 1 wherein said cytotoxic T cells are TALL-104 cells.

3. The method according to claim 1 wherein said cytotoxic T cells are TALL-103/2 cells.

4. A method of modifying a cytotoxic T cells while retaining the cytotoxicity of the cells comprising the steps of:

(a) culturing said cells in an effective amount of IL-2;

(b) culturing the IL-2 stimulated cells in an effective amount of IL-15 and

(c) optionally repeating steps (a) and (b) a selected number of times, wherein said modified cells demonstrate a change in at least one characteristic selected from the group consisting of increased proliferation,

differentiation, growth, phenotype, adhesion molecule expression, biodistribution, cytokine production profile, level of cytotoxic activity and tumor target spectrum.

5. The method according to claim 4 wherein said cytotoxic T cells are TALL-104 cells.

6. The method according to claim 5 wherein said cytotoxic T cells are TALL-103/2 cells.

7. A method of modifying TALL-104 cells comprising:

culturing TALL-104 cells in an effective amount of IL-15, wherein said cells grow at a rate faster than when stimulated by IL-2, and have an altered phenotypic, cytotoxic and cytokine profile.

8. The method according to claim 7 wherein said modified cells have an increased level of cytotoxicity.

9. The method according to claim 7, wherein said modified cells demonstrate a change in a characteristic selected from the group consisting of increased proliferation, differentiation, growth, phenotype, adhesion molecule expression, biodistribution, cytokine production profile, and tumor target spectrum.

10. The method according to claim 9 wherein said cytokine profile comprises increased expression of a cytokine selected from the group consisting of IL-10, GM-CSF, TNF-α and TNF-β

11. The method according to claim 9 wherein said cytokine profile comprises decreased expression of gamma interferon.

12. The method according to claim 9 wherein said phenotype comprises increased expression of the cytotoxic adhesion/activation marker CD56.

13. The method according to claim 9 wherein said phenotype comprises decreased expression of the adhesion molecule CD38.

14. A method for increasing the levels of cytotoxic activity and spectrum of tumor target recognition and growth of TALL-103/2 cells comprising culturing TALL-103/2 cells in an effective amount of IL-15, wherein said cells grow at a rate faster and have an expanded target cytotoxicity than when stimulated by IL-2.

15. The method according to claim 14, wherein said modified cells demonstrate a change in a characteristic selected from the group consisting of increased proliferation, differentiation, growth, phenotype, adhesion molecule expression, biodistribution, cytokine production profile, and tumor target spectrum.

16. Modified cytotoxic T cells produced by stimulating said cells in an effective amount of IL-15.

17. The cells according to claim 16 selected from the group consisting of TALL-104 cells and TALL-103/2 cells.

18. Modified cytotoxic T cells produced by the method of claim 1.

19. The cells according to claim 18 selected from the group consisting of TALL-104 cells and TALL-103/2 cells.

20. Modified cytotoxic T cells produced by the method of claim 4.

21. The cells according to claim 20 selected from the group consisting of TALL-104 cells and TALL-103/2 cells.

TALL-104

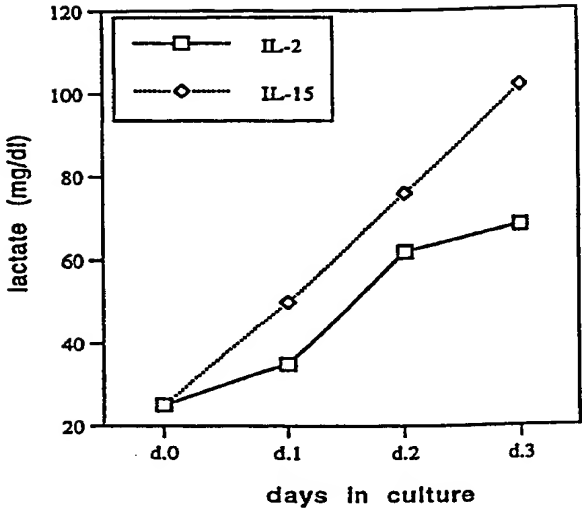


Fig. 1

TALL-104

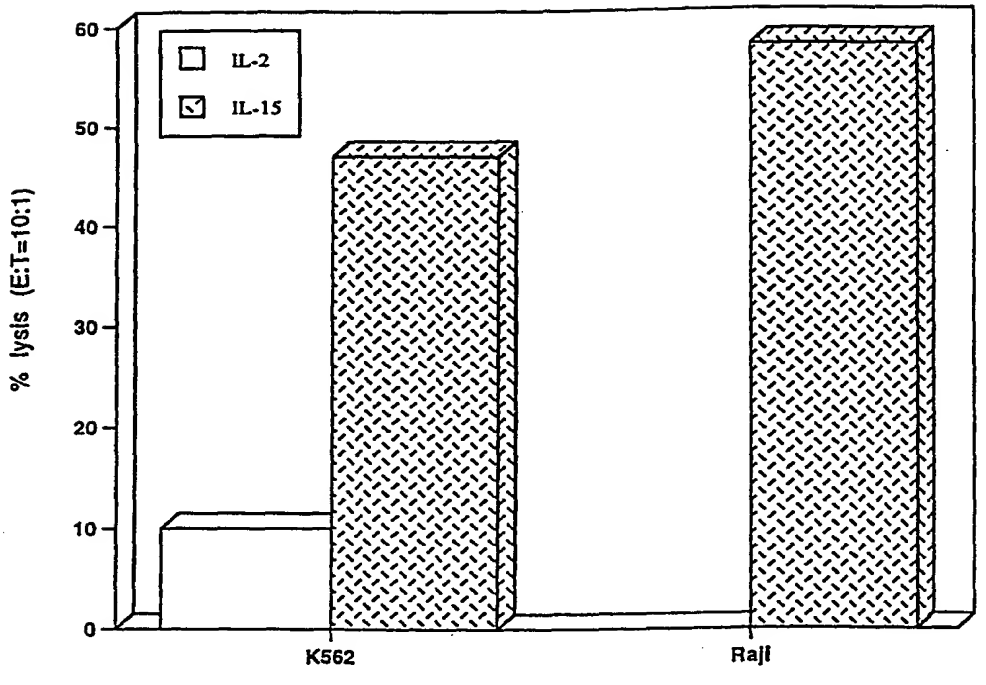


Fig. 2

TALL-104

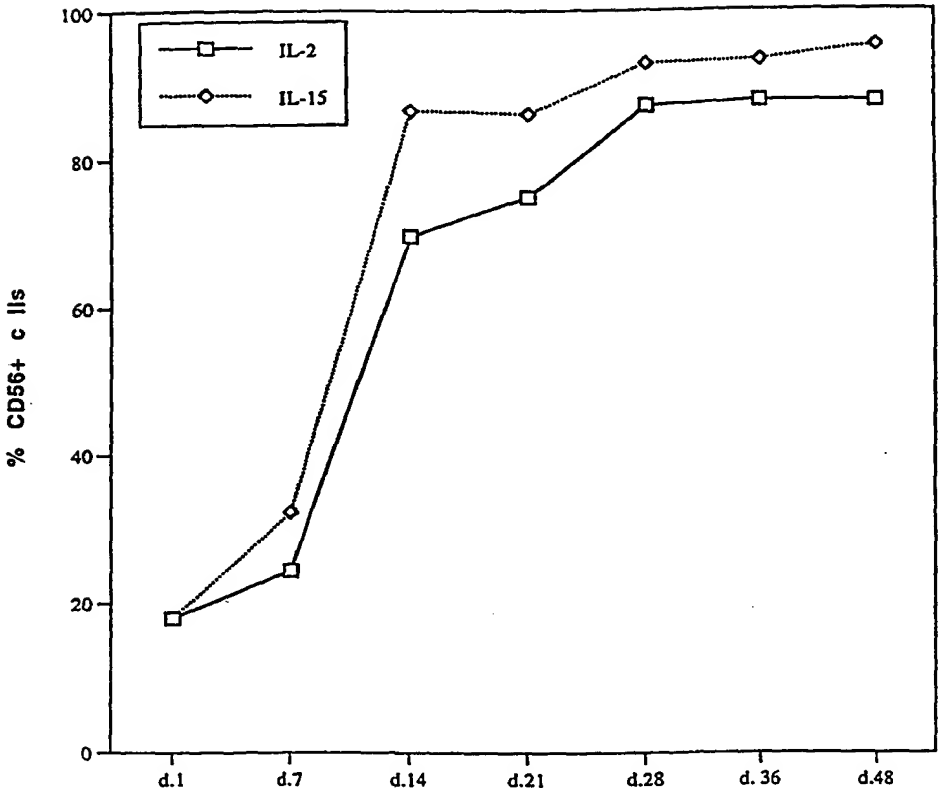


Fig. 3

TALL-104

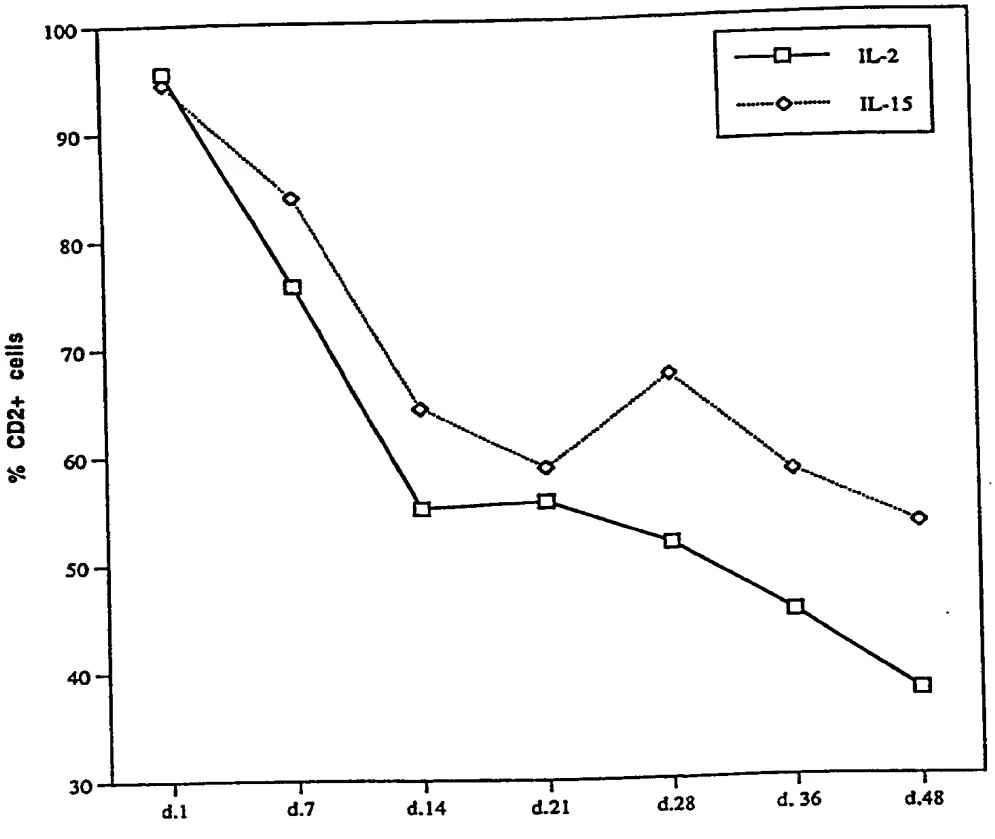


Fig. 4

TALL-104

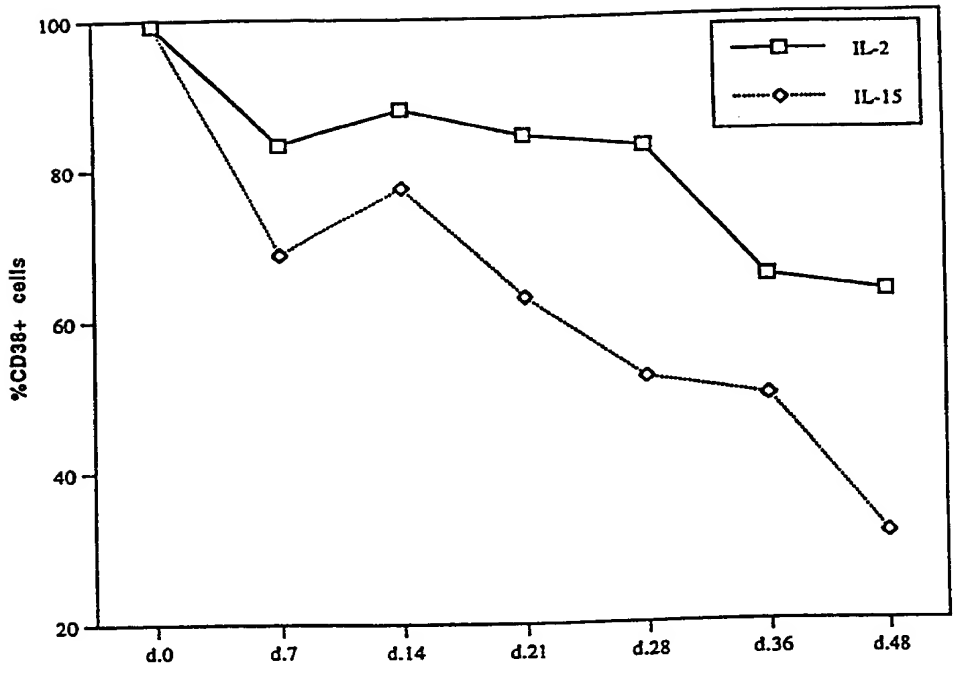


Fig. 5

TALL-104

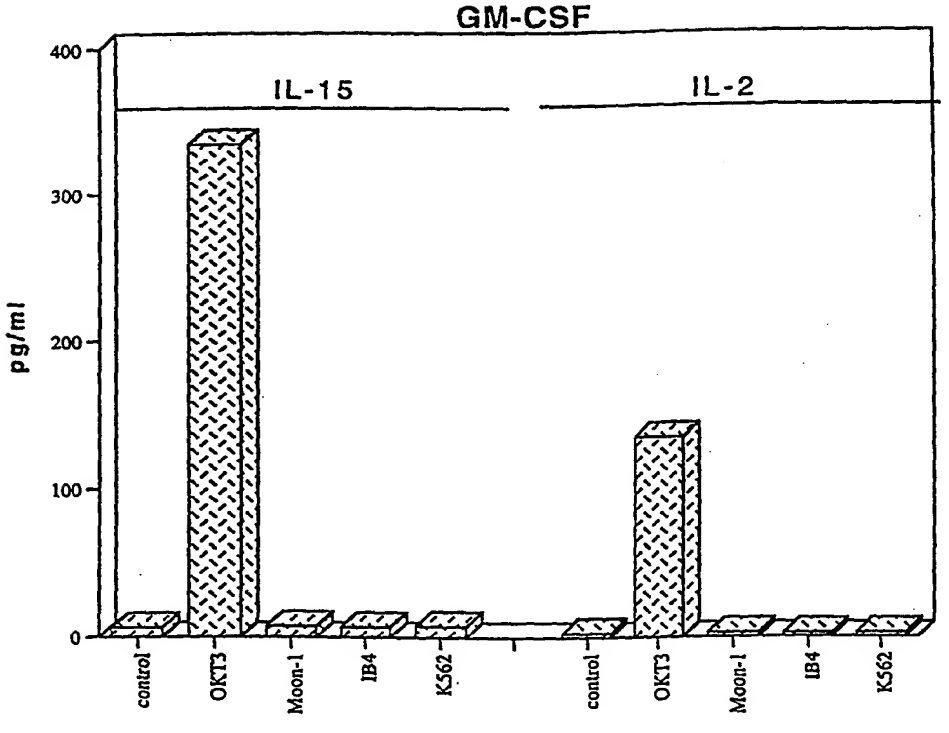


Fig. 6

TALL-104

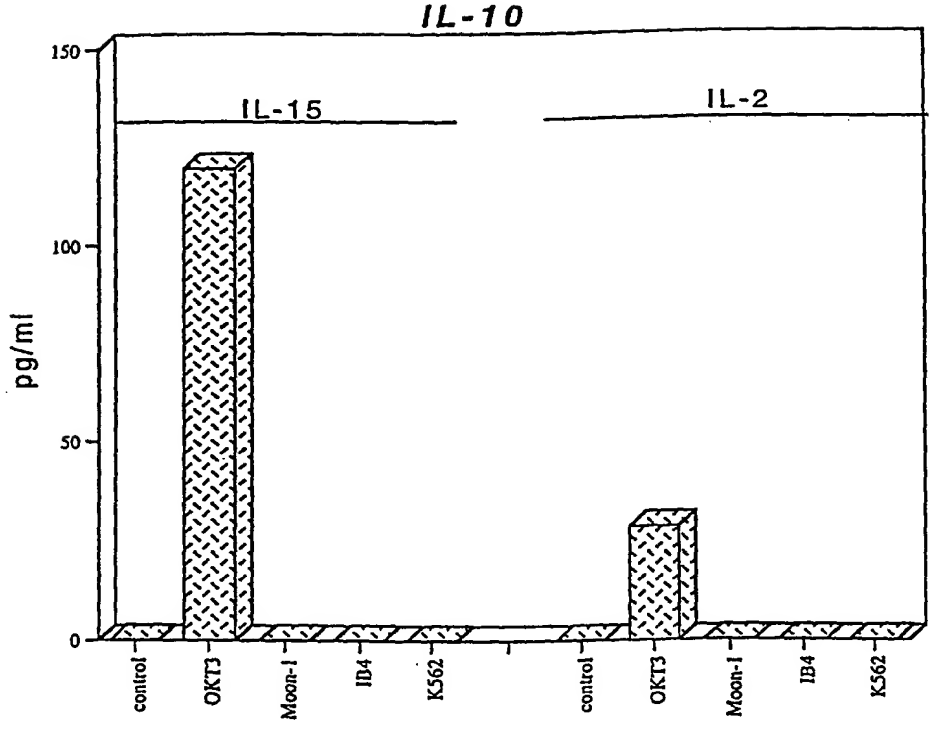


Fig. 7

TALL-104

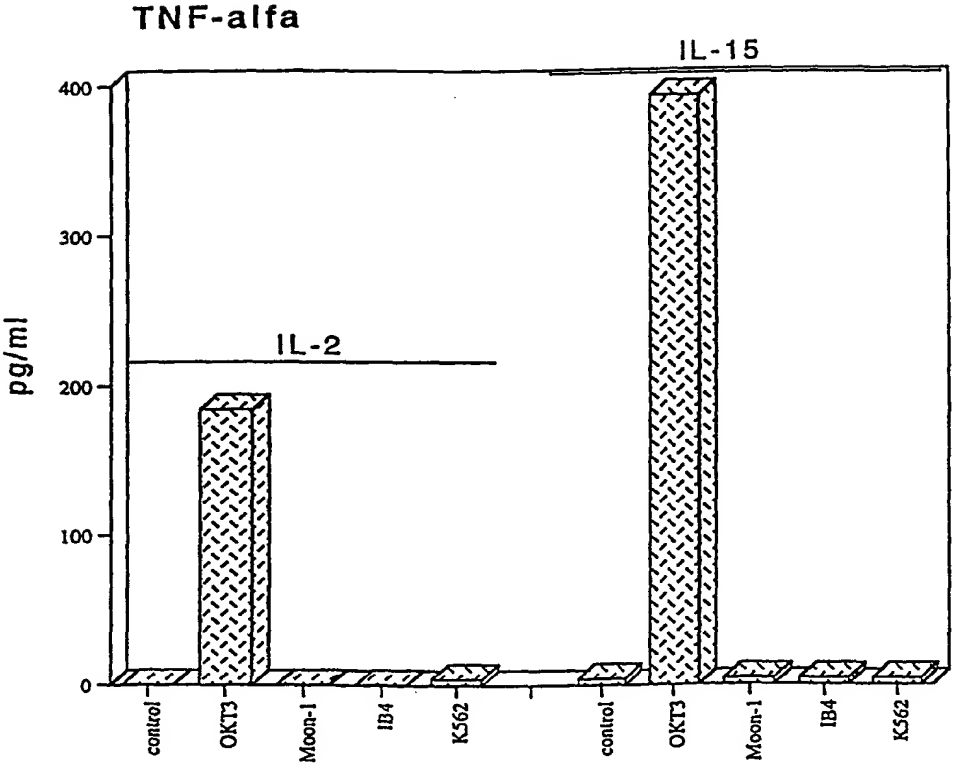


Fig. 8

TALL-104

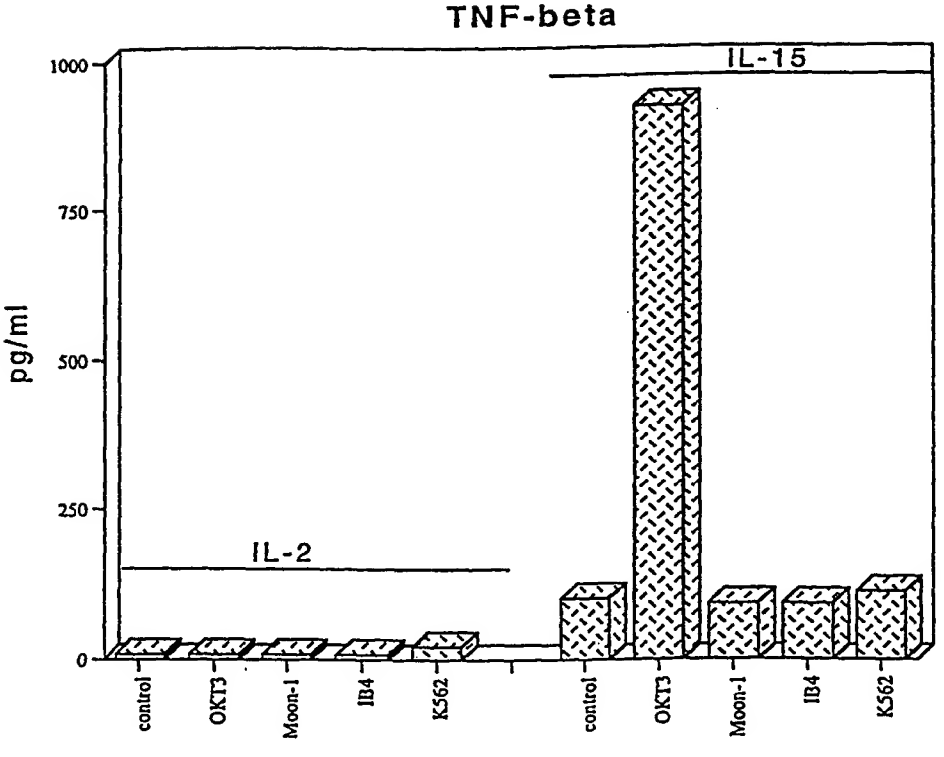


Fig. 9

TALL-104

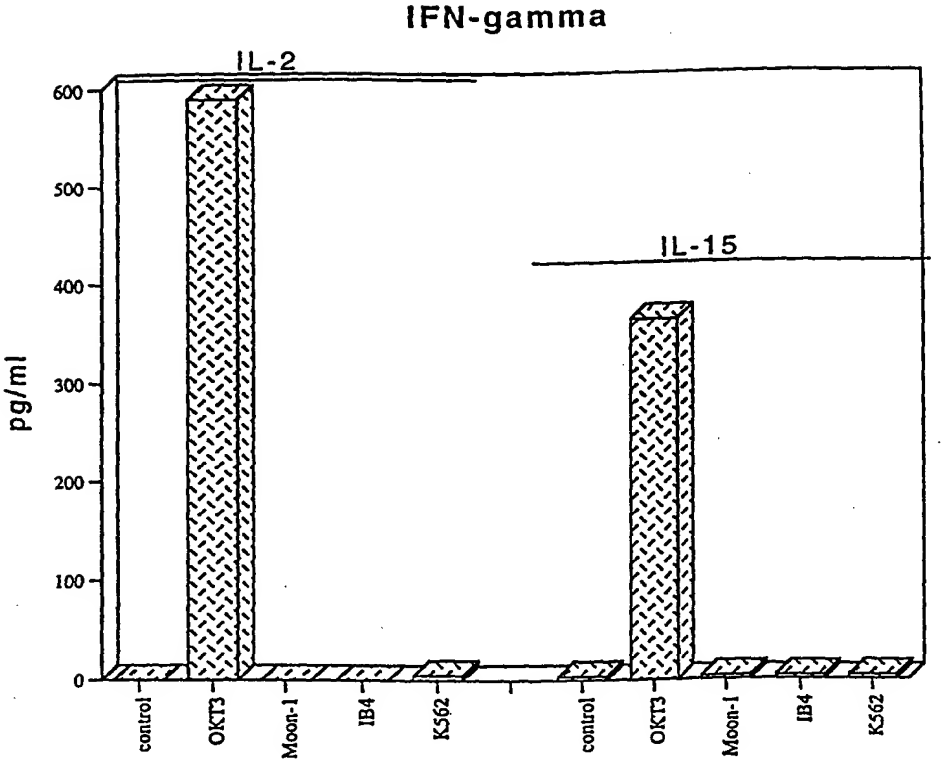
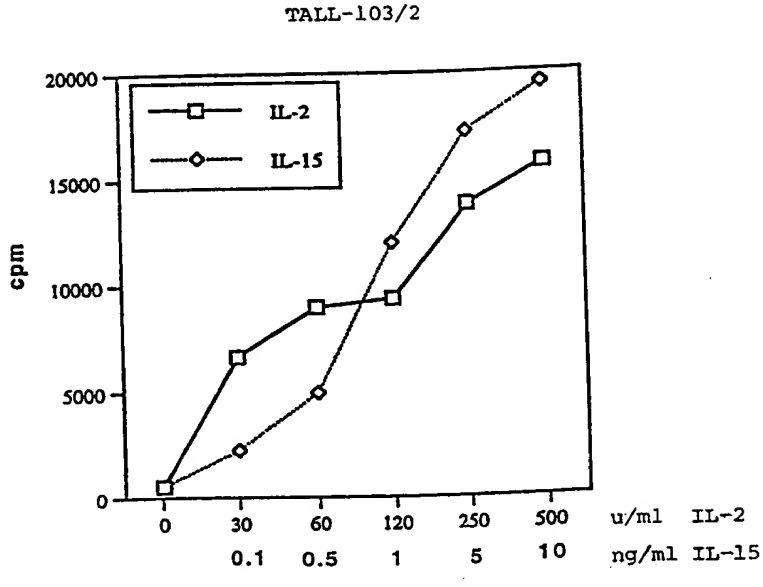


Fig. 10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10000
5000
0
cpm
IL-2
IL-15
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500
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0.1
0.5
1
5
10
ng/ml IL-15

Fig. 11

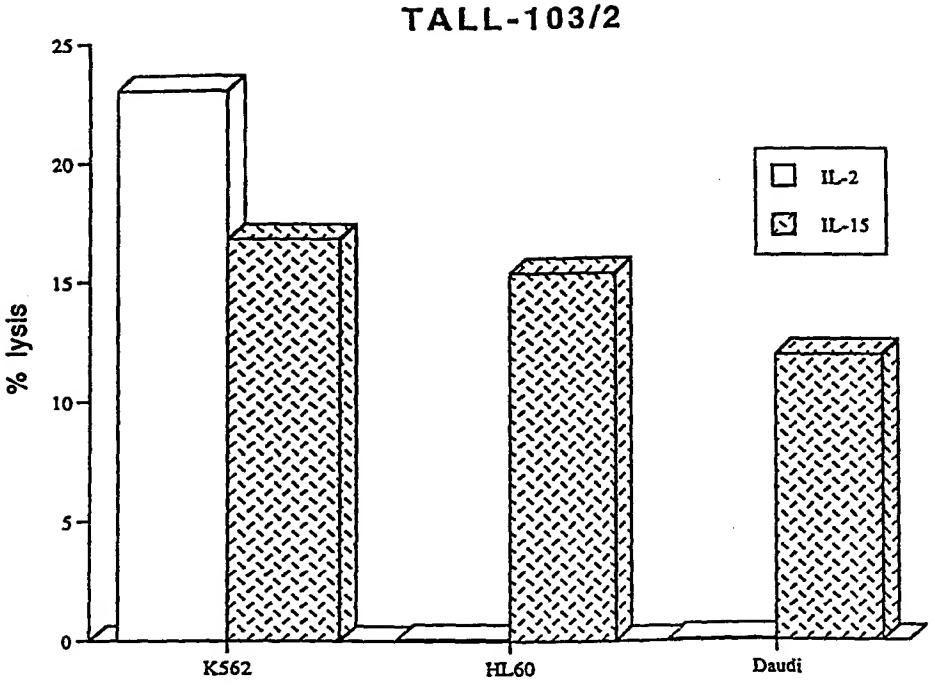
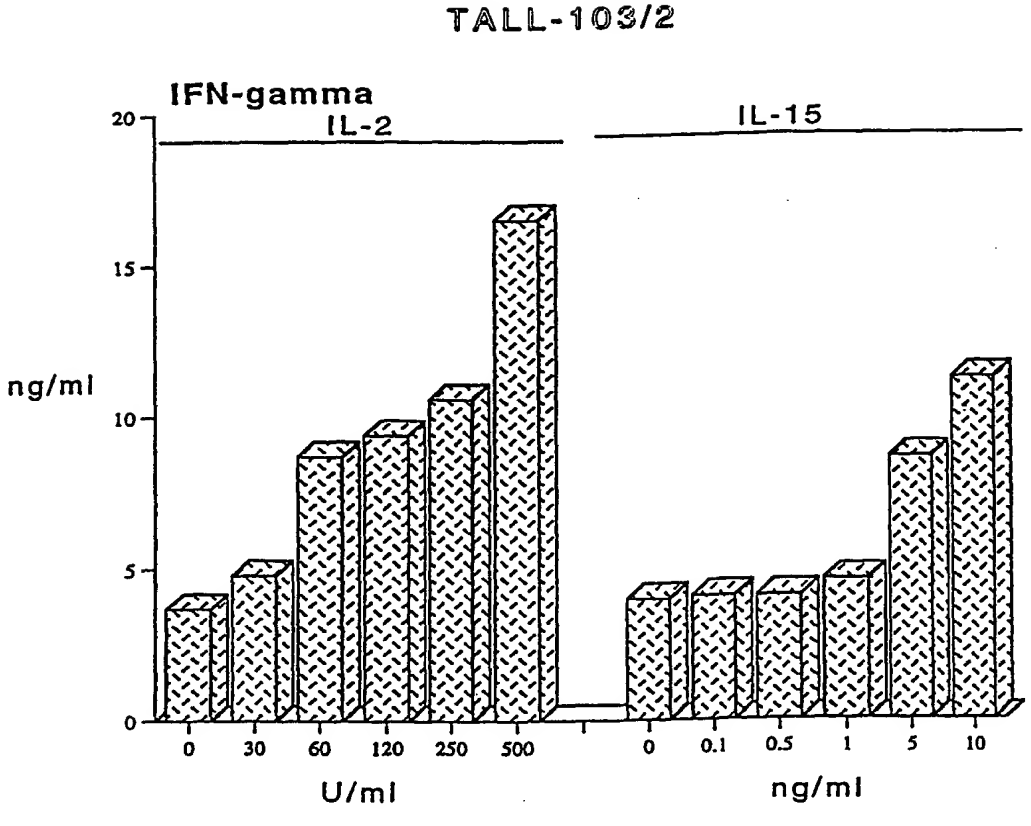


Fig. 12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IL-15
ng/ml
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15
10
5
0
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30
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120
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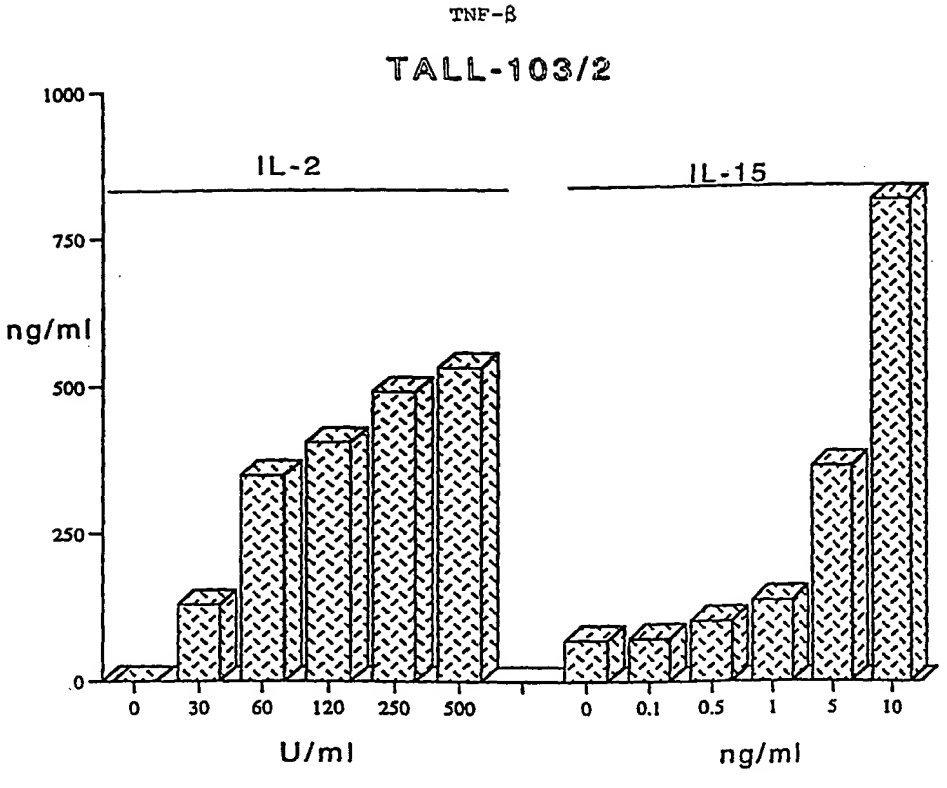
500
U/ml
0
0.1
0.5
1
5
10
ng/ml

Fig. 13


TNF-β
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IL-15
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500
250
0
ng/ml
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60
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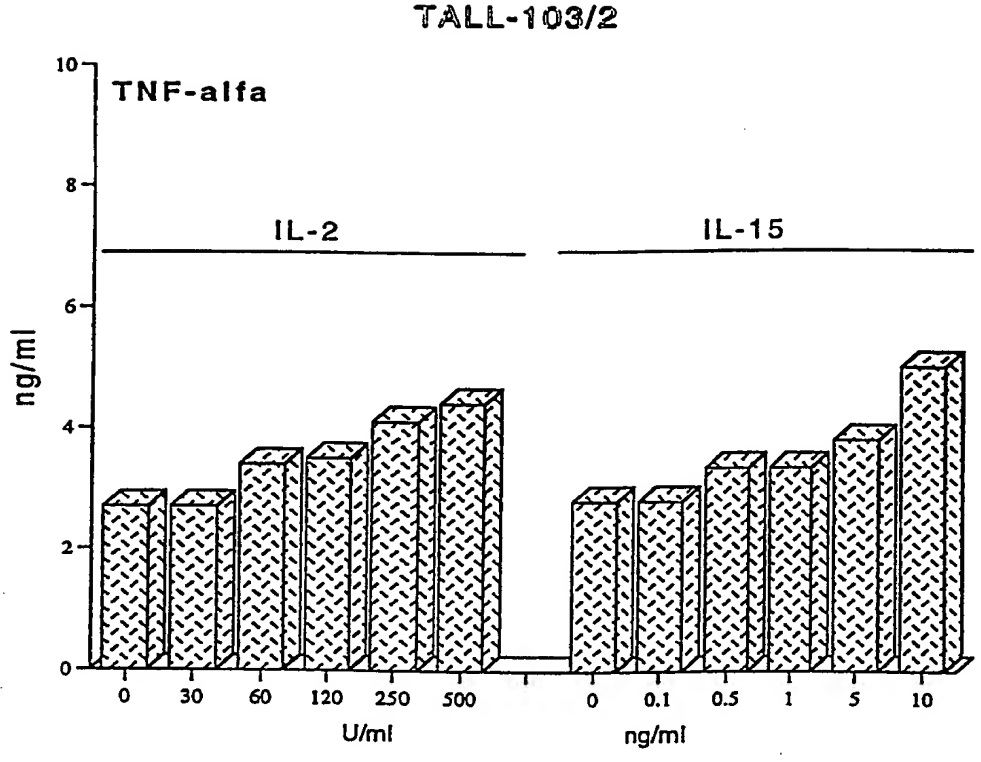
U/ml
0
0.1
0.5
1
5
10
ng/ml

Fig. 14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6
4
2
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U/ml
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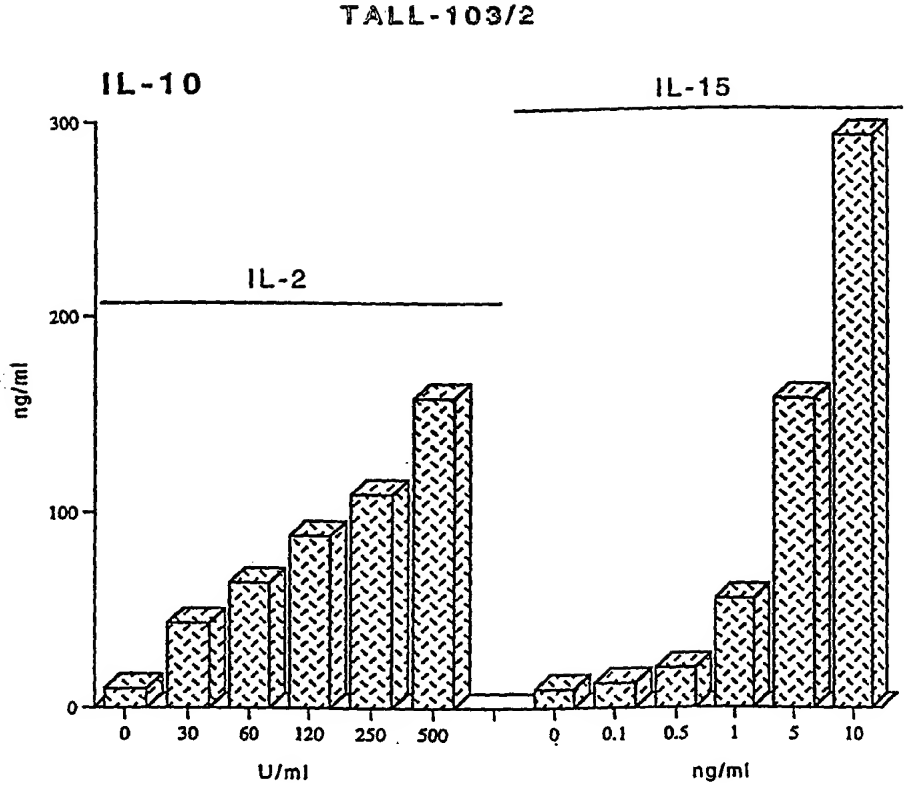
0.1
0.5
1
5
10
ng/ml

Fig. 15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IL-2
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ng/ml
0
30
60
120
250
500
U/ml
0
0.1
0.5
1
5
10
ng/ml

Fig. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04548

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 5/00, 5/02
US CL :435/375, 377; 530/351

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/375, 377; 530/351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,820,856 A (SANTOLI ET AL) 13 October 1998(13.10.98), see entire document.	1-21
A	US 5,702,702 A (SANTOLI ET AL) 30 December 1997(30.12.97), see entire document.	1-21
A	US 5,683,690 A (SANTOLI ET AL) 04 November 1997(04.11.97), see entire document.	1-21
A	WO 94/26284 A1 (THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY) 24 November 1994(24.11.94), see entire document, especially claims 1 and 2.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 APRIL 2000	Date of mailing of the international search report 27 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARIANNE DIBRINO Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998) ★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04548

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GAMERO et al. Interleukin 15 Induction of Lymphokine-activated Killer Cell Function against Autologous Tumor Cells in Melanoma Patient Lymphocytes by a CD18-Dependent, Perforin-related Mechanism. Cancer Research. 01 November 1995, Vol. 55, pages 4988-4994, especially Abstract and Introduction.	1-21
A	CESANO et al. TWO UNIQUE HUMAN LEUKEMIC T-CELL LINES ENDOWED WITH A STABLE CYTOTOXIC FUNCTION AND A DIFFERENT SPECTRUM OF TARGET REACTIVITY ANALYSIS AND MODULATION OF THEIR LYTIC MECHANISMS. In Vitro Cell. Dev. Biol. September-October 1992, Vol. 28A, pages 648-656, especially Summary, Introduction and Materials and Methods.	1-21
A	CESANO et al. Effects of lethal irradiation and cyclosporin A treatment on the growth and tumoricidal activity of a T cell clone potentially useful in cancer therapy. Cancer Immunol Immunother. 1995, Vol. 40, pages 139-151, especially Abstract.	1-21
A	SANTOLI et al. SYNERGISTIC AND ANTAGONISTIC EFFECTS OF IL-1a AND IL-4, RESPECTIVELY, ON THE IL-2-DEPENDENT GROWTH OF A T CELL RECEPTOR-gd+ HUMAN T LEUKEMIA CELL LINE. The Journal of Immunology. 15 June 1990, Vol. 144, No. 12, pages 4703-4711, especially Abstract.	1-21
A	DISANTO. Cytokines: Shared receptors, distinct functions. Current Biology. 01 July 1997, Vol. 7, No. 7, pages R424-R426, see entire article.	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04548

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST 1.2, STN(MEDLINE, EMBASE, BIOSIS, CAPLUS, SCISEARCH)
search terms: culture, IL-15, IL-2, interleukin(s), Santoli, Daniela Cesano, Alessandra, CTL, cytotoxic T lymphocyte(s), TALL-104, TALL-103, CD38, IL-10, GM-CSF, TNF, CD56, differentiation, phenotype, cytokine(s)